J. Physiol. (1971), **216**, *pp.* 659–682
With 8 text-figures
Printed in Great Britain

CATECHOLAMINE SECRETION BY THE ADRENAL MEDULLA OF THE FOETAL AND NEW-BORN FOAL

BY R. S. COMLINE AND MARIAN SILVER

From the Physiological Laboratory, University of Cambridge, Cambridge CB2 3EG

(*Received 8 March 1971*)

SUMMARY

1. The content and output of adrenaline and noradrenaline from the equine adrenal medulla has been investigated under different conditions in foetuses, foals and adult mares.

2. In the foetus only small amounts of both amines were secreted in response to stimulation of the peripheral ends of the splanchnic nerves to the gland; during anoxia the adrenal discharge was far greater and was independent of any nervous mechanism.

3. Whereas in the ruminant a direct adrenal response to low P_{O_2} is confined to the noradrenaline cells during foetal life only, the adrenal medulla of the foetal foal secreted both adrenaline and noradrenaline during asphyxia, and the direct response persisted for some days after birth. Noradrenaline was the amine predominantly released during asphyxia in the foetus.

4. Catecholamine output from the equine adrenal medulla changed with age, in that there was a gradual increase in both the absolute and relative amount of adrenaline released, irrespective of the stimulus applied, although at any given stage of development a higher proportion of adrenaline was secreted in response to stimulation of the splanchnic nerves than during anoxia.

5. The relative proportions of the two amines in the effluent blood bore little resemblance to those found in the glands, removed after prolonged asphyxia, in either foetuses or foals. Preliminary observations have indicated that more noradrenaline is present in the glands when the foetus remains relatively undisturbed within the uterus.

6. The possible significance of the larger adrenal response to asphyxia in the foetal foal in comparison with other species is discussed in relation to the development of the innervation and the growth of the adrenal cortex.

INTRODUCTION

The foetal adrenal medulla of widely different species contains a higher proportion of noradrenaline than that of the adult, but its capacity to secrete catecholamines has been investigated directly in only two species, the lamb and the calf (Comline & Silver, 1961, 1966). In both an unusual property was observed, namely the ability of the foetal medullary cells, particularly those releasing noradrenaline, to respond directly to anoxia. In the lamb, the splanchnic nerves to the gland play an increasing role in the adrenal discharge during anoxia near term and the direct response is lost before parturition, whereas in the calf the non-nervous mechanism predominates until birth and then disappears rapidly.

In the present investigation the activity of the adrenal medulla before and after birth has been examined in the foal, which during foetal life differs from the ruminant in two major respects. First, the greater efficiency of its placenta, particularly for gaseous exchange, means that the foetus develops at a higher overall P_{O_2} (Comline & Silver, 1970) and secondly, the umbilical cord remains patent throughout parturition, thereby allowing transfer of placental blood to the new-born foal (Rossdale, 1967). Under these conditions it seemed possible that the adrenal medulla might be even more sensitive than those of the foetal ruminants to changes in P_{O_2}, possibly by means of a neural mechanism. In fact the results have shown that a massive response of the chromaffin cells to anoxia, in which both adrenaline and noradrenaline are secreted, overshadows any response mediated by the splanchnic nerves. This property is retained, with some regression, for several days after birth.

METHODS

Many of the procedures and techniques were developed for the study of the adrenal in the foetal lamb and calf (Comline & Silver, 1961, 1966; Comline, Silver & Silver, 1965) and the investigation of the equine placenta (Comline & Silver, 1970): only essential information, modifications and additional procedures are described in detail below.

Animals. The experiments were carried out on small pony mares, the gestational age of which was known to within ± 3 weeks (term $\sim$ 340 days); and on new-born and suckling foals up to the age of 3 months. Food, but not water, was withheld from the adult animals for 24 hr before the experiments.

Anaesthesia. The animals were anaesthetized by the intravenous infusion of either chloralose (initial dose up to 70 mg/kg I.V.) or sodium pentobarbitone (12 g/100 ml. in 0·9 % (w/v) NaCl). Details of the effects of these two anaesthetics on blood gas tensions and pH in the mare are given elsewhere (Comline & Silver, 1970).

Operative procedures. In the majority of experiments, after exposure and catheterization of uterine and umbilical vessels, blood samples were taken at intervals (Comline & Silver, 1970), beginning $2\frac{1}{2}$–3 hr after the induction of anaesthesia, to

follow conditions in both mother and foetus during subsequent preparation. When necessary, supplementary O_2 or positive pressure ventilation was given to the mare. The foetus had to be removed from the uterus for the preparation of the adrenal gland, and haemorrhage from the incision was controlled by electrocoagulation. The foetus was kept warm and the cord protected by enclosing it within the membranes.

Adrenal effluent blood was collected from the left gland after removal of the left kidney in all animals whether foetal, new-born or adult. In the horse the adrenal gland lies on the superior mesenteric artery to which it is closely adherent. The main adrenal vein arises at the posterior pole and follows a variable course to the inferior

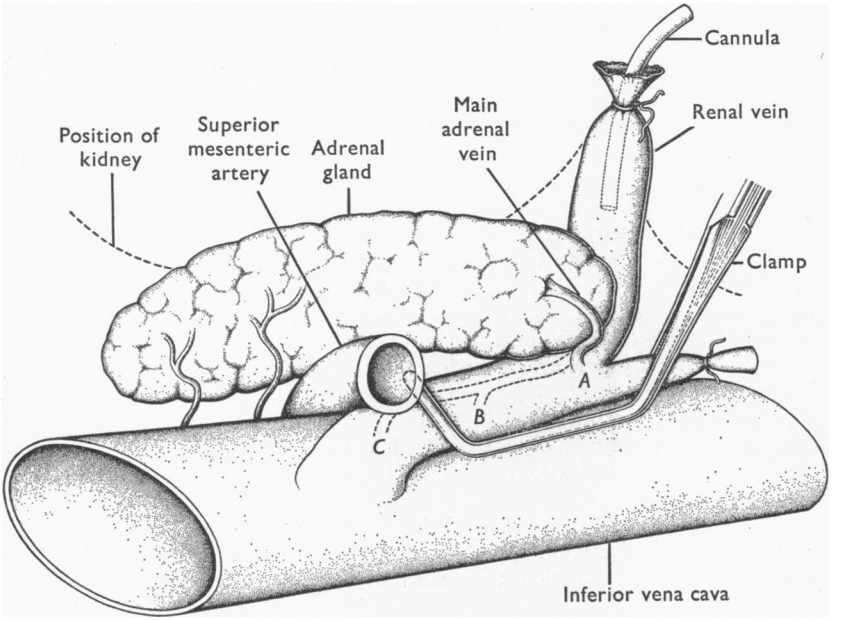

Fig. 1. Diagram to indicate variations (*A*, *B*, *C*) in the venous drainage from the equine adrenal gland and to show the position of the clamp to divert the main adrenal venous outflow from *A* or *B* into the renal vein catheter.

vena cava (Fig. 1). In most animals it appeared to join the renal vein at a point level with the superior mesenteric artery (B), in others it remained completely separate from the renal vein and both entered the vena cava anterior to the artery (C), while in a few animals the adrenal vein drained into the renal vein close to the gland itself (A). In those animals in which the venous junction was accessible a clamp was placed below it (Fig. 1), in a manner similar to that used for the calf and lamb, to divert adrenal blood back into a catheter placed in the renal vein. This procedure was impracticable when the adrenal vein drained directly into the vena cava. In these animals small bore polythene or vinyl tubing was inserted directly into the adrenal vein itself, which inevitably placed some restriction on the adrenal venous outflow compared with preparations in which blood was collected from a renal venous pocket. It was impracticable to collect the entire venous effluent from the gland, since additional small veins which drain the anterior and central parts (Fig. 1) could not be cannulated or diverted into a large vessel.

Cannulae were also inserted into the trachea and into the femoral vein and artery; in some experiments, the left splanchnic nerves were sectioned and the peripheral ends prepared for stimulation. In one foetus and four foals the spinal cord was pithed according to the technique used for the lamb (Comline & Silver, 1961). Adrenal effluent blood samples were collected during the insertion of the probe and in the foal positive pressure ventilation from a Starling-Ideal pump was begun as soon as spontaneous breathing stopped. The animals were then left for a 30–40 min recovery period during which the foals were given an infusion of 500 ml. Dextran solution to restore blood pressure.

All animals were heparinized (1000 i.u./kg, foals and adults; 2000 i.u./kg, foetuses), and blood pressure was recorded continuously after the operative procedures had been completed.

Asphyxia and anoxia. The first period of asphyxia was produced in the foetuses by clamping the umbilical cord, and in the foals either by allowing them to rebreathe N_2 or 10 % CO_2 in N_2 from a balloon or by positive pressure ventilation with these gas mixtures.

When two tests were carried out on the same animal the first period of asphyxia was restricted to 4–5 min and both the foetuses and foals were then resuscitated with O_2 and maintained with positive pressure ventilation if respiration did not occur spontaneously; the second test was then applied after an interval of 20 min. Provided that blood pressure, pH and P_{O_2} were maintained throughout, there was no evidence that the second adrenal response to anoxia was any less than the first.

Experimental design. Because of the shortage and expense of the animals the following tests were generally performed during each experiment, provided the conditions remained stable:

1. Resting adrenal output.
2. The effect of asphyxia or anoxia with splanchnic nerves intact. Duration: 4–5 min followed by resuscitation, section of the splanchnic nerves and a rest period of about 20 min.
3. The resting output after section of the splanchnic nerves.
4. The effect of stimulation of the peripheral ends of the splanchnic nerves: 2 tests, each of 1–3 min duration, followed by rest period of 20–30 min.
5. Resting output.
6. The effects of asphyxia or anoxia (splanchnic nerves cut). Duration: until death of animal 10–20 min later.

In some animals only test 2 was performed and the total response (10–20 min) was followed. In others tests 3–6 were performed and in the adult animals and one foetus only tests 3 and 4 were carried out.

Blood samples: collection, treatment and measurement. P_{O_2}, P_{CO_2} and pH in both maternal and foetal blood were monitored at $\frac{1}{4}$–$\frac{1}{2}$ hourly intervals; in the foals samples were taken less frequently as conditions remained more stable. Arterial samples were taken immediately before a period of asphyxia and at 2 min intervals throughout the ensuing test period, beginning 1 min after the onset of asphyxia. Standard Radiometer (Copenhagen) equipment was used for all P_{O_2}, P_{CO_2} and pH analyses.

Adrenal effluent blood samples were collected during rest periods (2–5 min), during electrical stimulation (30/sec; 20–30 V; 1 msec square-wave pulses) of the splanchnic nerves (1–3 min collection after a lag of 15–30 sec) and during asphyxia and anoxia (consecutive samples at 1, 2 or 3 min intervals, depending on the rate of blood flow through the gland). The subsequent treatment and analysis of the samples has been given previously (Silver, 1960; Comline & Silver, 1961, 1966).

The output of catecholamines from the left adrenal gland is expressed as μg base secreted/gland/min and the content is given as mg base/g wet wt. All results are corrected for losses during extraction and chromatography.

RESULTS

Catecholamine content of adrenal glands

The right adrenal gland was used for the determination of catecholamine content since this remained undisturbed during the course of the experiments. However, some catecholamine depletion was inevitable, particularly in the foetus, since the glands were removed 5–7 hr after the induction of anaesthesia from foetuses which were exteriorized and asphyxiated.

The values, summarized in Fig. 2, showed a clear change both in content and proportion of adrenaline between 10 days and 3 months of age. The percentage of adrenaline in the gland of the oldest group of foals was similar to that of the adult; the lower catecholamine concentration in the adult glands probably reflects the greater amount of adrenal cortical development relative to medullary growth, since the mean total content per gland was 12·9 mg in the adult and 13·6 mg in the 3 month old animals.

In the perinatal period there was little change in catecholamine content and no rise in the proportion of adrenaline. The apparent drop in concentration immediately after birth, which in this small group of animals was only statistically significant for adrenaline ($P < 0{\cdot}01$), suggests that some adrenal medullary discharge may well occur during parturition.

In view of previous findings on the effects of operative trauma and asphyxia on adrenal catecholamine content in the lamb foetus (Comline & Silver, 1961) it seemed important to compare present findings with observations on undisturbed non-asphyxiated foals *in utero*. Accordingly, in four additional mares near term the foetal adrenal glands were removed during the course of experiments under chloralose anaesthesia in which the foetus remained *in situ* and in good condition for 3–4 hr. The total catecholamine concentration of these glands was higher, and the proportion of adrenaline lower, than the corresponding values for asphyxiated foetuses of the same age (Table 1). These differences were due to the higher concentration of noradrenaline in the glands of the undisturbed foetuses; the adrenaline levels were similar in both groups.

Adrenal medullary secretion

'*Resting*' *conditions*

Table 2 shows the mean catecholamine output from the left adrenal gland in six groups of animals under resting conditions. The amounts of both amines released under the conditions of these experiments varied greatly; levels were highest in those animals in which the adrenal vein was cannulated directly or when the clamp was poorly positioned, thereby restricting flow. Deterioration of the preparation, e.g. circulatory collapse

or impairment of umbilical blood flow, led to an abnormally high output from the gland; these values have been omitted from the series given in Table 2. There was, however, a considerable amount of individual variation within each group, although a fairly consistent change occurred in the

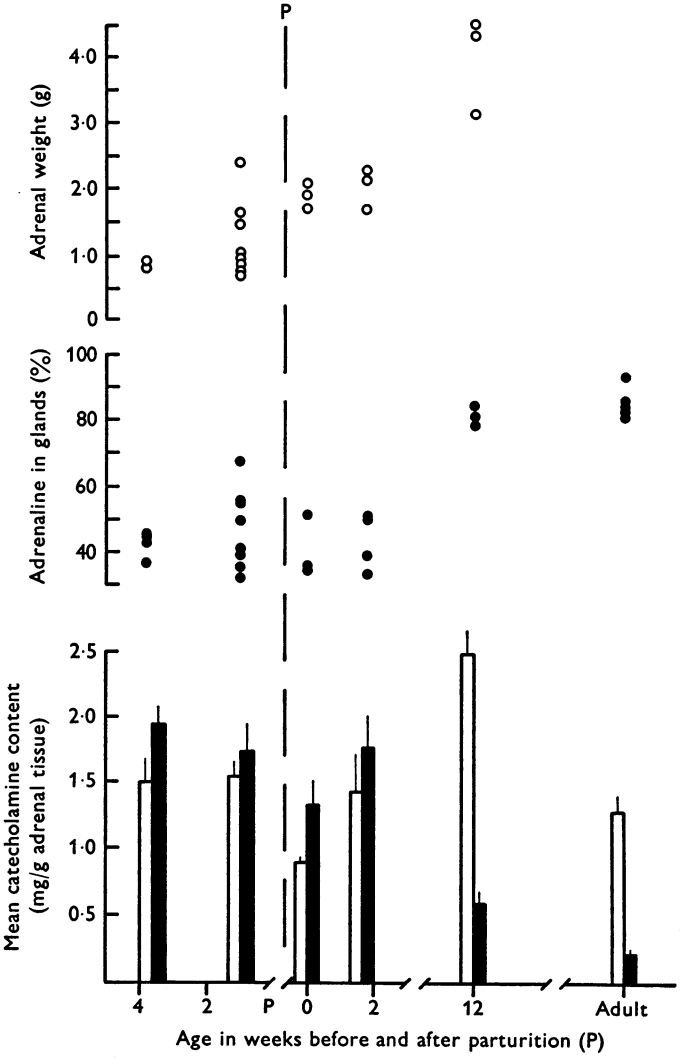

Fig. 2. Mean catecholamine content per g of adrenal tissue in the foal, at different stages before and after birth, and in the adult mare. □, adrenaline; ■ noradrenaline (vertical bars indicate S.E. of mean). Values for adrenal weights (○) in g, and the proportion of adrenaline (●) expressed as a percentage of the total catecholamine content on the adrenal glands of each animal are given above the appropriate histogram.

TABLE 1. A comparison of the catecholamine content of the adrenal glands, removed under different conditions, from two groups of foetal foals

Animals	No.	Duration of expt. (hr) before adrenalectomy	Gestational age (days)	Mean catecholamine content (mg/g wet wt. ± S.E.) Adrenaline		Noradrenaline		Adrenaline (%)	
Foetuses *in utero*	4	3–4	328–335	1·32 ± 0·20	N.S.	3·09 ± 0·27	$P < 0·01$	29·5 ± 1·4	$P < 0·01$
Foetuses exteriorized and asphyxiated	8	5–7	330–336	1·54 ± 0·13		1·76 ± 0·23		47·5 ± 4·2	

TABLE 2. Mean resting output of catecholamines (μg.gland.min ± S.E. of mean) from the equine adrenal medulla of foetal, young and adult animals

Age	Splanchnic nerves intact No.	Adrenaline	Noradrenaline	Splanchnic nerves cut No.	Adrenaline	Noradrenaline
Antepartum (gestation age)						
310–320 days	2	0·67	2·62	2	0·66	1·91
330–340 days	5	0·64 ± 0·41	1·11 ± 0·60	6	0·98 ± 0·20	1·98 ± 0·56
Post-partum						
New-born	3	0·78 ± 0·11	1·83 ± 0·78	5	1·11 ± 0·32	0·95 ± 0·50
10 days	1	0·77	0·30	2	1·72	0·85
3 months	5	0·49 ± 0·15	0·35 ± 0·16	4	1·49 ± 0·39	0·38 ± 0·16
Adult	—	—	—	3	1·43 ± 0·37	0·38 ± 0·07

proportion of noradrenaline secreted with age; it was highest in the foetuses and lowest in the 3 month old foals and adults.

After the splanchnic nerves had been cut the resting output from the gland either remained unchanged or even increased, despite the fact that a recovery period of 15–20 min elapsed before the second series of resting samples was collected. Trauma or disturbance of the arterial supply to the gland during splanchnic nerve section might account for these findings. However, the levels found in all animals, save those in which overt anoxia had occurred, were only $\frac{1}{10}$–$\frac{1}{100}$ of the maximum discharge obtainable from the gland during different forms of stimulation.

There was an over-all increase in resting adrenal blood flow with age (Table 3) but since this was accompanied by a rise in adrenal weight of similar magnitude the flow/g of tissue showed no consistent change.

Effects of splanchnic nerve stimulation

The data obtained from foetuses, young foals and adults are summarized in Fig. 3. There was a gradual increase in the total catecholamine output from the adrenal glands with age. The calculated values for catecholamine output/kg body wt. min, which are given in parentheses above each histogram, show that maximal nerve stimulation of the adrenal medulla results in a discharge which almost keeps pace with the growth of the animal.

Most of the increase in total catecholamine secretion was due to a rise in adrenaline output. In fact the percentage adrenaline secreted in response to stimulation was higher than the proportion of this amine present in the glands (Fig. 2) at all ages tested. This discrepancy may be even greater, since the values for the proportion of adrenaline given in Fig. 2 were obtained from glands removed at the end of the experiments in which significant noradrenaline losses had occurred (Table 1).

Only a small number of animals was available at any given age group and there was considerable individual variation as indicated by the S.E.S of the means (Fig. 3); samples taken during two or more periods of stimulation in any one animal were however more uniform. The amounts secreted under chloralose anaesthesia tended to be higher than those in animals anaesthetized with sodium pentobarbitone, but this was not a consistent finding. It seems probable that the extent of the discharge elicited both by splanchnic nerve stimulation and by asphyxia depended to a much greater degree upon the depth of anaesthesia, on the general condition of the preparation and on the completeness of the adrenal effluent blood collection than on the type of anaesthetic *per se*.

There was a small rise in adrenal blood flow during splanchnic nerve stimulation (Table 3) associated with the rise in blood pressure which occurred under these conditions. In some cases, however, when the flow was

TABLE 3. Changes in blood flow through the left adrenal gland of the foal with age; under resting conditions, during splanchnic nerve stimulation (Stim.) and during anoxia

No. of animals	Age (days)	Mean wt. of left adrenal gland (mg ± S.E.)	Mean left adrenal blood flow (ml/min ± S.E.)						Mean flow/g adrenal tissue.min		
			n	Resting*	*n*	Stim.	*n*	Anoxia†	Resting	Stim.	Anoxia
12 foetuses	310	0·714	3	0·65 ± 0·19	1	0·90	3	2·02 ± 0·29	0·91	1·26	2·83
	335	0·941 ± 0·112	9	1·17 ± 0·23	7	1·89 ± 0·58	8	2·59 ± 0·53	1·24	2·01	3·01
13 foals	0–1	1·720 ± 0·359	4	1·18 ± 0·17	4	2·20 ± 0·66	4	2·48 ± 1·02	0·69	1·28	1·44
	10	1·855 ± 0·209	4	2·40 ± 0·46	3	3·23 ± 1·20	3	2·73 ± 0·77	1·29	1·74	1·47
	3 months	4·130 ± 0·525	5	4·50 ± 1·49	5	6·42 ± 1·90	4	5·32 ± 0·40	1·09	1·55	1·29
3 adults		11·780 ± 0·435	3	8·07 ± 1·97	3	7·83 ± 1·09		—	0·69	0·67	—

* Values for each animal obtained from two to four observations.

† Maximal flow obtained during a period of anoxia (one to two observations per animal).

more than doubled, it seemed unlikely that this blood pressure rise was the entire explanation. The mean rise in adrenal blood flow in eight foetuses was only $0{\cdot}56 \pm 0{\cdot}30$ ml./min ($P > 0{\cdot}05$), whereas in the twelve foals in which the effects of splanchnic nerve stimulation were tested the mean increase of $1{\cdot}24 \pm 0{\cdot}41$ ml./min was statistically significant ($P < 0{\cdot}02$).

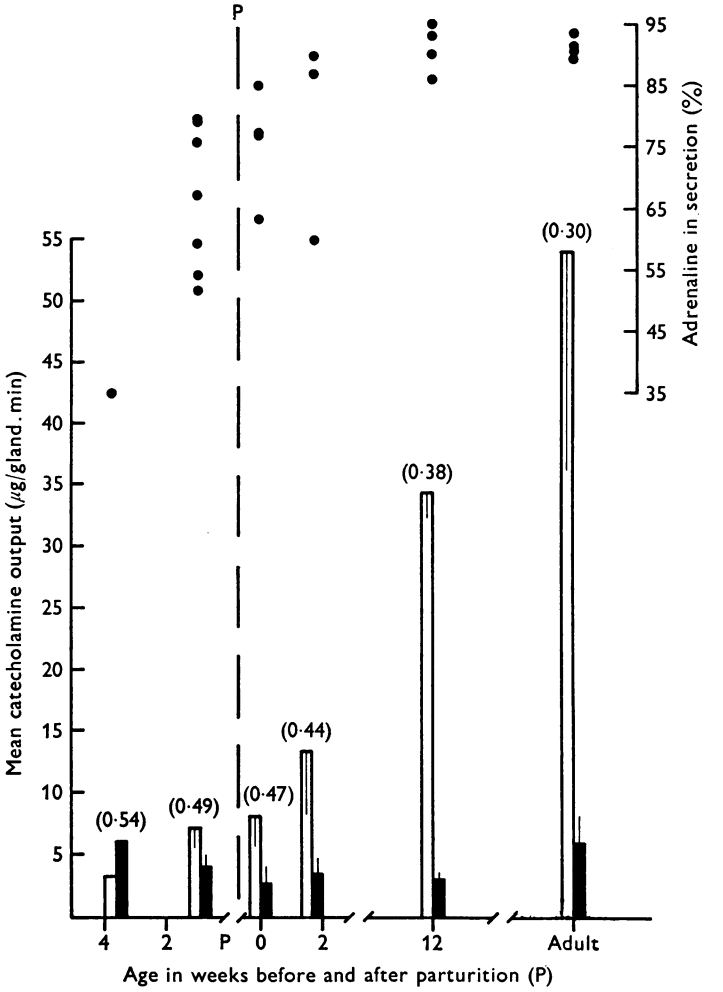

Fig. 3. Mean catecholamine output from the left adrenal gland during stimulation of the peripheral ends of the splanchnic nerves (30/sec; 20 V; 1 msec pulse width) in foetuses, foals and adults. ■, noradrenaline; □, adrenaline (vertical bars indicate the S.E. of mean); percentage adrenaline in the discharge from each animal tested (●) is given in the upper part of the Figure. The mean rate of catecholamine secretion/kg body wt. min is given in parentheses above each histogram.

The effects of asphyxia and anoxia

It was important that the animals were well oxygenated initially before the effects of anoxia or asphyxia were tested; blood pressure, pH and P_{CO_2} were also measured and maintained where possible, although during the lengthy preparation, particularly in the foetal experiments, the pH in both maternal and foetal blood inevitably fell.

Table 4 shows the results obtained immediately after the initial preparation of the mare and cannulation of the foetal vessels had been completed in experiments under chloralose anaesthesia. These values, obtained 2½–3 hr after the induction of anaesthesia and before surgical interference with the foetus itself, are comparable with data obtained from the conscious mare and foetus (Comline & Silver, 1970). Sodium pentobarbitone

TABLE 4. Mean values (± S.E.) for P_{O_2}, P_{CO_2} and pH in foetal and maternal blood 2½–3 hr after the induction of anaesthesia (chloralose) before removal of the foetus from the uterus in six experiments

	P_{O_2} (mm Hg)	pH	P_{CO_2} (mm Hg)
Maternal arterial blood	79·4 ± 8·2	7·385 ± 0·014	37·4 ± 1·97
Foetal arterial blood	34·0 ± 1·52	7·350 ± 0·009	45·5 ± 1·88

anaesthesia had, in our hands, a more deleterious effect on maternal acid–base balance than chloralose. This was shown in the previous study in which the effects of the two anaesthetics were compared (Comline & Silver, 1970). In the present experiments umbilical and uterine blood samples were taken from some of the mares under barbiturate anaesthesia before exteriorization of the foetus and the results confirmed the earlier findings.

In all animals adequate oxygenation was maintained throughout the period of preparation. Blood samples for analysis of P_{O_2}, P_{CO_2} and pH were taken before any tests were made on the effects of asphyxia or anoxia on catecholamine secretion; a summary of these initial values is given in Table 5.

Foetus. In three animals the effect of asphyxia in the presence and absence of the splanchnic nerves was tested in the same foetus. The results of two such experiments under sodium pentobarbitone anaesthesia are given in Fig. 4. In both animals the first period of asphyxia was induced with the splanchnic nerves intact, by occlusion of the umbilical cord. This led to a rapid fall of P_{O_2} to zero, a rise in P_{CO_2}, and a fall in pH. In the younger foetus (4 weeks before term; Fig. 4*a*) there was a tenfold increase in noradrenaline output accompanied by only a small rise in adrenaline secretion. In the foetus near term (Fig. 4*b*) the total output from the gland was much greater; adrenaline secretion had increased, but noradrenaline

TABLE 5. Mean values (± S.E.) for arterial P_{O_2}, P_{CO_2} and pH before and during asphyxia (foetuses) or anoxia (foals)

	1 min before occlusion of umbilical cord or N_2 ventilation			4–5 min after onset of asphyxia or anoxia		
No. of animals	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	pH	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	pH
10 foetuses	32·0 ± 2·4	64·8 ± 4·9	7·18 ± 0·017	2·6 ± 1·1	113·0 ± 9·3	7·02 ± 0·026
4 new-born	98·5 ± 14·7	34·9 ± 4·2	7·41 ± 0·056	6·3 ± 1·6	34·3 ± 8·2	7·44 ± 0·040
3, 10 days	107·0 ± 4·7	47·0 ± 5·5	7·34 ± 0·047	11·9 ± 3·6	42·0 ± 7·8	7·44 ± 0·036
4, 3 months	82·5 ± 6·4	49·8 ± 5·3	7·32 ± 0·014	7·9 ± 1·7	47·4 ± 13·0	7·37 ± 0·063

was still the major component of the discharge. In comparison the response to stimulation of the splanchnic nerves was small in both animals, but, whereas noradrenaline was the major component in the younger foetus, adrenaline was preferentially secreted near term. In fact in the older foetus the output of adrenaline during stimulation was similar to the maximum level obtained during asphyxia.

The effect of a second period of asphyxia was tested by positive pressure ventilation with 10% CO_2 in N_2 to simulate conditions after occlusion of the umbilical cord as nearly as possible. In the younger foetus there was no difference between the first and second response to asphyxia (Fig. 4*a*) despite the intervening denervation of the adrenal. A slight decline in absolute values was observed in the animal near term, but this foetus survived for only 3 min after the onset of asphyxia.

The data obtained in all experiments in which the effects of asphyxia were tested are summarized in Fig. 5, in which means have been calculated from maximum outputs obtained during the first 4–5 min asphyxia only, since this duration of sampling was common to all but one animal. Although insufficient numbers were available for any statistical treatment the results indicate that during foetal life the adrenal medulla can respond to asphyxia independently of its nerve supply. Indeed the mean values obtained with the nerves intact (Fig. 5*a*) were almost identical with those found after splanchnic nerve section (Fig. 5*b*) irrespective of the anaesthetic used, although the values obtained under chloralose were always higher than those found under sodium pentobarbitone. A similar divergence in the adrenal response to anoxia under these two anaesthetics also occurs in the calf (Comline & Silver, 1966).

No detailed analysis of the factors responsible for the asphyxial discharge of the adrenal medulla was made in the foetal foal. In previous studies on both lamb (Comline *et al.* 1965) and calf (Comline & Silver, 1966) the fall in P_{O_2} was strongly implicated. Table 5 shows the mean changes which occurred in the present experiments in the foetal blood during the first 4–5 min asphyxia; after this period the pH continued to fall and the P_{CO_2} to rise while the P_{O_2} remained at or near zero. The maximum discharge from the gland usually occurred between 6 and 10 min after the onset of asphyxia (see Fig. 7) and this was almost invariably accompanied by a rise in blood flow through the gland (Table 3). The increase in blood pressure which generally followed occlusion of the umbilical cord was not associated with any marked change in adrenal blood flow. In fact the adrenal response increased soon after the onset of bradycardia, a phenomenon which appears to be characteristic of foetal anoxia in many species (Dawes, 1968).

Foals. In the new-born and older foals the effects of anoxia or asphyxia

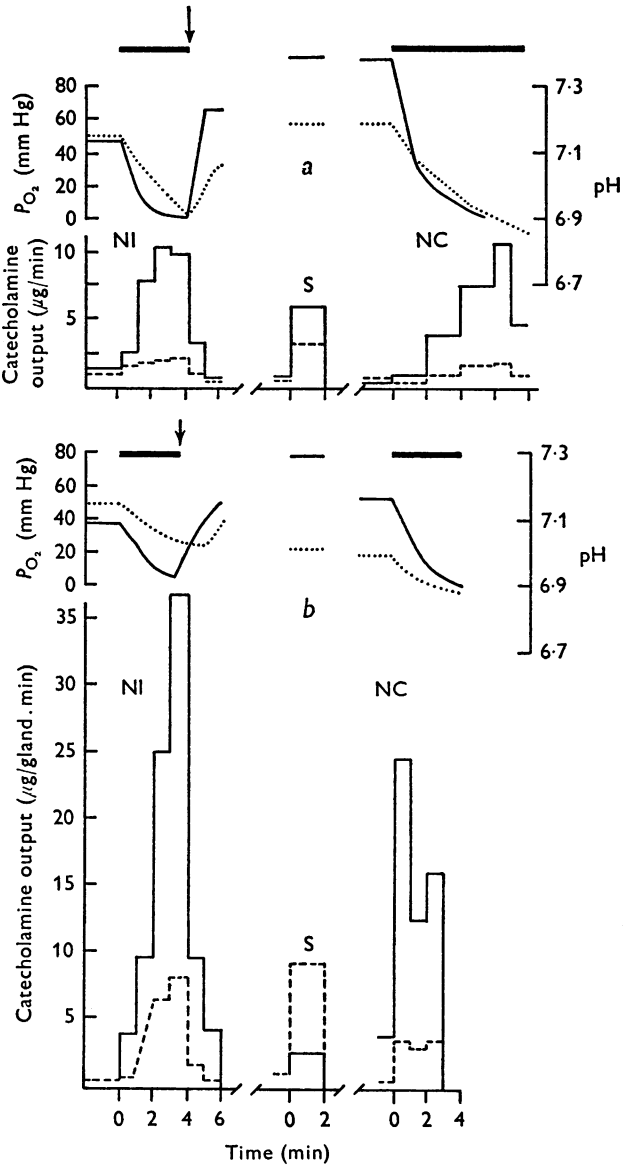

Fig. 4. The effects of asphyxia on the adrenal medullary discharge before and after denervation of the gland in two foetuses: *a*, 4 weeks before parturition; *b*, a few days before birth. In each Figure the changes in P_{O_2} (——) and pH (.....) are shown above and the outputs of noradrenaline (——) and adrenaline (– – –) during one or two min periods are given below. NI, splanchnic nerves intact; NC, splanchnic nerves cut; S, stimulation of the peripheral ends of the nerves (details in Fig. 2). ▬▬period of asphyxia; ↓ start of artificial ventilation.

were tested by artificial ventilation with N_2 alone or with 10% CO_2 in N_2 respectively. The latter was less well tolerated, and so N_2 ventilation was used in the majority of experiments, especially when the effects of the presence and absence of the splanchnic nerves on the adrenal response were to be tested in the same animal.

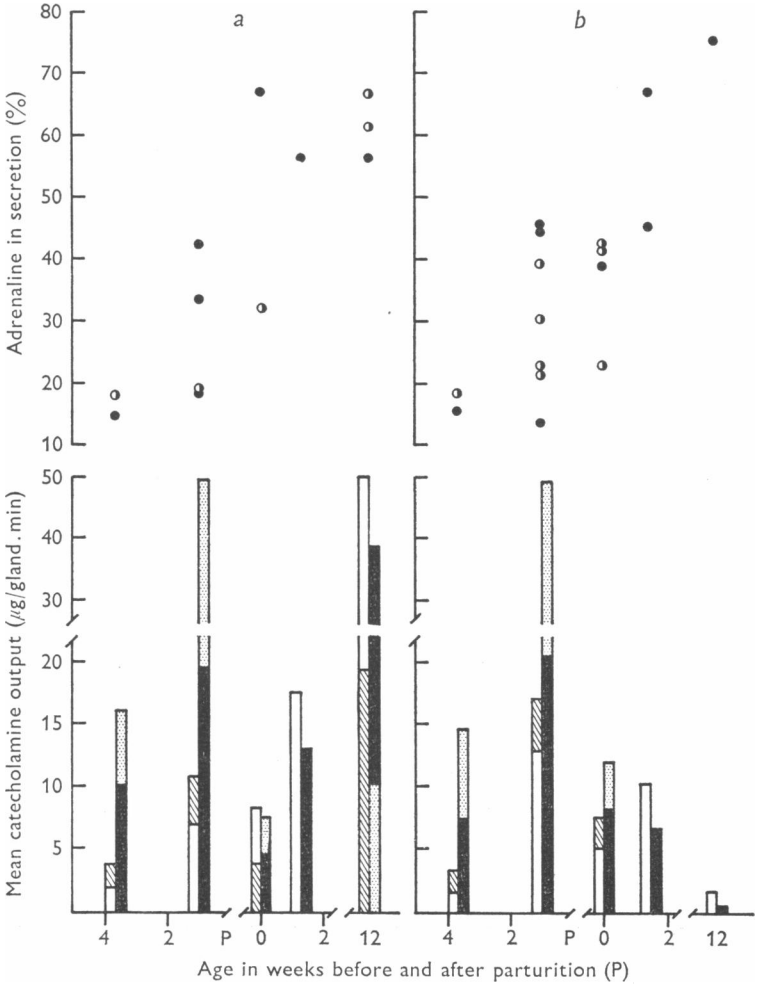

Fig. 5. Mean maximum catecholamine output during 4–5 min asphyxia or anoxia in foetuses and young foals, with splanchnic nerves (*a*) intact and (*b*) cut. Noradrenaline output under chloralose, ▦, and sodium pentobarbitone anaesthesia, ■. Adrenaline output under chloralose, ▧, and sodium pentobarbitone, □. The percentage adrenaline in the discharge in each animal anaesthetized with chloralose (◑) or sodium pentobarbitone (●) is given above each histogram.

Four new-born foals were used and the results from 1 of these are shown in Fig. 6. The foal, which was a few hours old, was anaesthetized with sodium pentobarbitone. The adrenal response to anoxia with the nerves intact was small in this animal, and the discharge contained a high proportion of adrenaline. A high percentage of adrenaline was also secreted

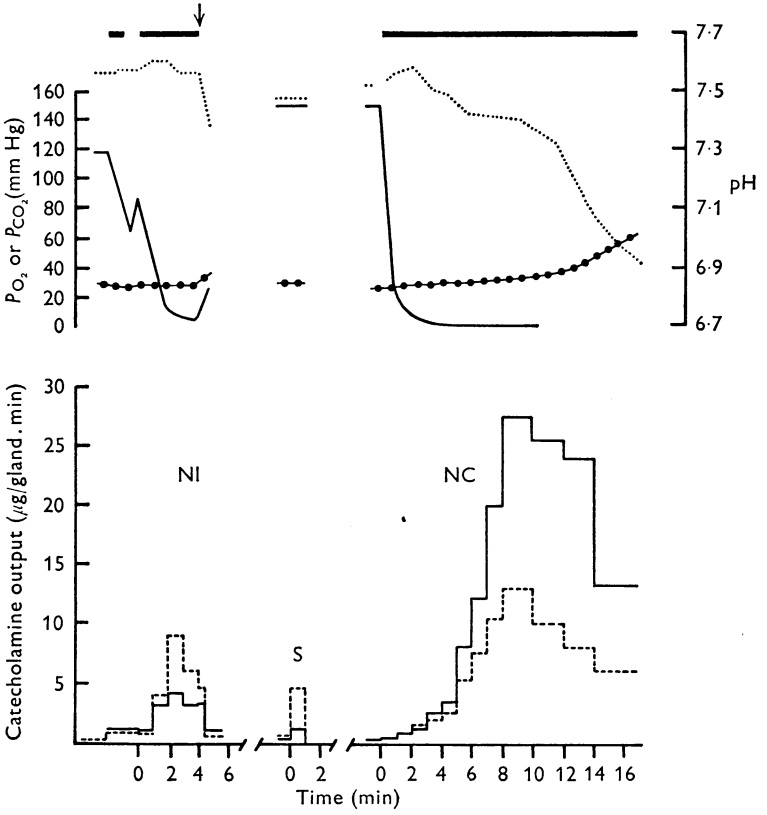

Fig. 6. The effects of anoxia on the adrenal medullary discharge before and after denervation of the gland in a new-born foal. Above: changes in P_{O_2} (——) pH (.....) and P_{CO_2} (● – ●). Below: output of noradrenaline (——) and adrenaline (– – –) during 1, 2 or 3 min periods. ▬▬, period of anoxia by artificial ventilation with N_2; ↓, O_2 given. Other symbols as in Fig. 4.

in response to splanchnic nerve stimulation. During the second period of anoxia, however, in which the adrenal response was slow in onset, the output gradually increased and eventually a massive discharge occurred, of which about 60 % was noradrenaline. This last response was comparable with that found in the later term foetus (Fig. 4*b*).

The relatively poor initial response of the adrenal medulla to anoxia

in all the new-born animals, whether the gland was innervated or not, is shown in Fig. 5, in which the mean maximum levels attained during the first 4–5 min of anoxia may be compared with the much larger values found in the foetus. The presence or absence of the splanchnic nerve supply to the gland again appeared to have little effect on the response at this stage.

The maximum adrenal medullary output attained in any of the new-born foals (during prolonged anoxia) was also lower than that found in the foetus. Values of 27 and 21 μg noradrenaline/min were obtained in foals under sodium pentobarbitone and chloralose anaesthesia respectively, while the corresponding figures for the late-term foetus were 37 and 111 μg noradrenaline/min.

The relatively small discharge from the adrenal medulla of the new-born foal compared with that in the foetus may be due partly to the slower onset of anoxia after birth and also to a probable depletion of the gland during parturition. The low catecholamine content of the glands at this time (Fig. 2) lends support to this view.

The first definitive evidence for a nervous component in the adrenal response to anoxia was obtained at 10 days after birth (Fig. 5). In two animals anaesthetized with sodium pentobarbitone the total catecholamine discharge from the intact gland was double that found immediately after birth and the amount of adrenaline released was now far greater than at earlier stages of development. After section of the splanchnic nerves there was still a response from the gland during anoxia; this again consisted of the secretion of more adrenaline than noradrenaline, but, unlike the foetus and new-born animal, the discharge was maintained for only 4–5 min at most, after which rapid circulatory collapse led to death of the animal.

At 3 months after birth the discharge of the denervated gland in response to anoxia was virtually lost; the results from one animal are shown in Fig. 5. The discharge with intact nerves was extremely variable in this group, particularly the results obtained from three animals under sodium pentobarbitone anaesthesia. In two of these the adrenal response did not appear to be initiated during the 4 min period of anoxia although stimulation of the splanchnic nerves to the gland later resulted in the usual discharge. In the other animal (shown in Fig. 5) the highest output of the group was obtained. The values obtained under chloralose anaesthesia were lower than those found under sodium pentobarbitone.

Further analysis of the response of the denervated adrenal medulla

The fact that the adrenal medulla of the foal appeared to respond directly to asphyxia for some days after birth was in marked contrast to findings in both lamb and calf, and so merited further investigation.

Although all visible splanchnic nerve connexions to the adrenal gland were cut in the present experiments, the possibility could not be excluded that other minor nervous pathways to the gland remained intact and functional. This was investigated firstly by the effect of hexamethonium and secondly by destruction of the spinal cord by pithing to eliminate all nervous stimulation of the gland.

Hexamethonium

In the foetal lamb hexamethonium (10 mg/kg) blocks the effects on the adrenal medulla of stimulation of the peripheral ends of the splanchnic nerves, while the direct response of the gland to asphyxia is unchanged (Comline & Silver, 1961). Attempts to repeat this type of experiment in the foal met with little success. In two near-term foetuses the administration of hexamethonium (10 mg/kg) resulted in such a drastic fall in systemic blood pressure that the foetus became hypoxic and an adrenal discharge began before the umbilical cord could be clamped. The effect of hexamethonium block on the adrenal innervation could not therefore be tested by direct stimulation of the nerves, but the widespread circulatory collapse indicated that other parts of the sympathetic system had been blocked. Since hexamethonium had such a profound effect on the blood pressure of the exteriorized foetus near term, its use was abandoned and the effects of pithing were examined instead.

Pithing

The discharge from the adrenal medulla during anoxia was examined under chloralose anaesthesia after the destruction of the spinal cord in one foetus and four foals between 0 and 10 days of age. During and after the insertion of the probe there was a variable degree of stimulation of the adrenal medulla which continued for about 5 min. The maximum rates of output observed under these conditions are given in Table 6. The animals were then left to recover for 20–30 min.

The adrenal medullary response observed in the foetus and new-born foal is shown in Fig. 7, together with comparable data from a near-term foetus with intact adrenal innervation under chloralose anaesthesia. The similarity of the asphyxial discharge from the glands of these three animals is immediately apparent. All showed a massive secretion of noradrenaline with a smaller output of adrenaline, and in all the maximum discharge was attained after 7–10 min. The fact that virtually identical responses were obtained whether the stimulus employed was anoxia or asphyxia gives further indication that it is the fall in P_{O_2} which is the most likely factor concerned in the local response of the adrenal medullary cells in this species as well as in the lamb and the calf.

Foals between 5 and 10 days of age were far more susceptible to the effect of anoxia after pithing than the foetus or new-born animal. Despite the infusion of dextran solution to restore blood pressure after destruction of the spinal cord, and the adjustment of O_2 intake to maintain arterial P_{O_2} at 80–100 mm Hg, two foals succumbed within 2 min, and the remain-

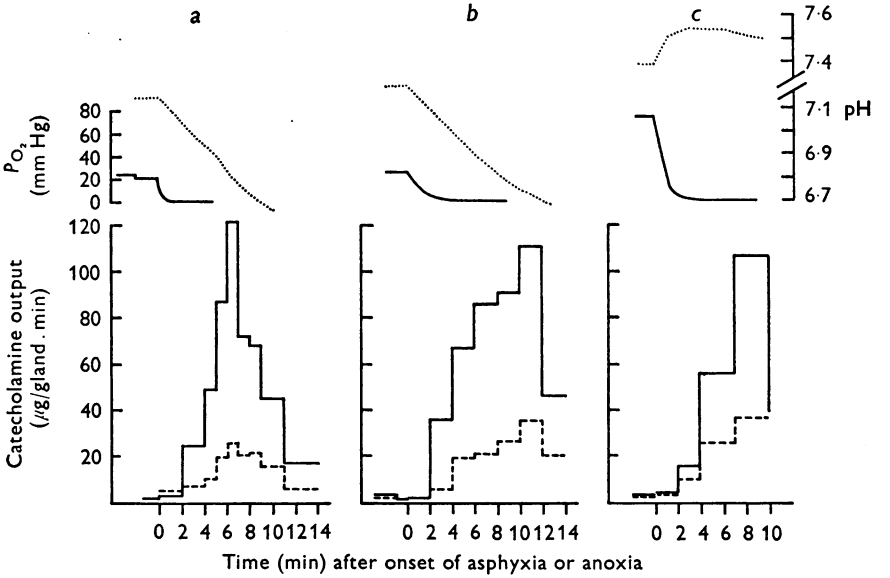

Fig. 7. The effects of umbilical cord occlusion (*a*, *b*) and ventilation with N_2 (*c*) on the adrenal medullary discharge in three animals under chloralose anaesthesia; *a*, near term foetus with splanchnic nerves intact; *b*, near term foetus with spinal cord destroyed by pithing; *c*, new-born foal with spinal cord destroyed by pithing, and maintained by positive pressure ventilation. Symbols as in Figs. 4 and 6.

TABLE 6. The change in catecholamine output during pithing and subsequent anoxia in the foetus and foal after birth

	Adrenaline (A) and noradrenaline (N) output (μg/min)					
	Pithing*		Resting†		Anoxia‡	
Animals	A	N	A	N	A	N
Foetus (near term)	1·1	5·3	0·06	0·50	15·7	67·5
New-born foal	3·5	9·0	0·93	1·37	25·6	56·8
10-day foal	1·2	1·5	0·04	0·07	0·7	2·1

* Maximum output during 5 min following pithing of the spinal cord.
† Output immediately before anoxia.
‡ Maximum output during the first 3–5 min anoxia.

ing 10 day old foal survived only 3–4 min anoxia. The maximum adrenal medullary discharge from this animal was negligible compared with that in the pithed foetus or new-born foal (Table 6) and was also smaller than the output obtained after 4–5 min anoxia from 10 day old animals with cut splanchnic nerves (Fig. 5*b*). A comparable adrenal output might have occurred if the pithed animals had survived the anoxia for 5 or more min, but, in view of the difficulties of maintaining the circulation under these conditions, further experiments seemed unjustified.

DISCUSSION

There are certain differences between the responses of the adrenal medulla of the foetal and new-born foal and those of the lamb and calf. First, the magnitude of the total asphyxial discharge of the medulla is particularly striking in the foal at the end of gestation. The mean maximum output near term was 30 μg/gland.min under sodium pentobarbitone anaesthesia, and more than twice this amount was secreted under chloralose; in the foetal calf, which is similar in size and weight, the corresponding figure was 15 μg/gland.min in animals under barbiturate anaesthesia. This difference in the secretory capacity of the adrenal medulla of the two species may in fact be even greater, since it is unlikely that the whole of the adrenal venous outflow was collected in all the present experiments owing to the diffuse anterior venous drainage of the adrenal gland of the horse.

Secondly, although noradrenaline was the major component in the direct response of the foetal adrenal medullary cells to asphyxia in all three species, in the foal comparatively large amounts of adrenaline were also released by this mechanism, particularly at term and after birth. This was in marked contrast to the ruminants in which the secretion of significant amounts of adrenaline was closely associated with the development of the neurally induced asphyxial discharge.

Finally, the loss of this characteristically foetal response was relatively slow in the foal; a small direct effect of anoxia was detectable even at 10 days after birth. This was very different from the rapid decline in the direct sensitivity of the cells of the calf adrenal medulla within 15 h of birth and the virtual disappearance of the direct response some days before parturition in the sheep foetus.

The results obtained in the present limited series of experiments on the foal were sufficiently clear cut to compensate for the difficulties in working with these animals. The cost and availability of suitable animals restricted the number which could be examined at any given stage of development. Differences in the duration of the experiments, the type and depth of anaesthesia, the condition of the animals and the anatomy of the adrenal

circulation, may also have contributed to the variation of the absolute values obtained during both anoxia and nervous stimulation. Nevertheless, the technique has the advantage that it gives a direct measurement of catecholamine output under different conditions, which could not be predicted from the variation in the concentration of catecholamines stored in the gland. Indeed the present results in the foal, and the more complete series in the foetal lamb and new-born calf (Comline & Silver, 1961, 1966), indicate that the catecholamine content of the gland bears little relation to the composition of the secretion unless the changes are very large.

Further comparison of the results from the foetal lamb, calf and foal shows that the amount and composition of the discharge from the adrenal medulla at the end of gestation depends on the interplay between various factors. In the foetal lamb, the neural mechanism for asphyxial discharge largely supersedes the local response of the cells immediately before parturition. On the other hand the splanchnic innervation in both the calf and foal would certainly seem to be inefficient and probably immature at birth. In both, the position resembles the development of the somatic innervation in certain other species. Deficiencies in the innervation of skeletal muscle, including a more diffuse sensitivity to acetylcholine before the formation of discrete myoneural junctions, have been reported in both the new-born rat and kitten (Diamond & Miledi, 1962; Buller, 1969; Redfern, 1970), and a similar sensitivity to acetylcholine occurs in the adrenal medulla of the foetal and new-born calf (Silver, 1960; Comline & Silver, 1966). In the calf at least the neural and local effects of hypoxia potentiate each other and similar effects probably occur even in the lamb near term, for the asphyxial discharge is always slightly greater than that found on direct stimulation of the splanchnic nerves (Comline & Silver, 1961). In the foal the greater sensitivity of the adrenal medullary cells to the direct effect of asphyxia completely overshadows any neural mechanism during maximal stimulation of the gland. On the other hand, a response to stimulation of the peripheral ends of the splanchnic nerves can be elicited in the foetus as well as in the new-born foal. The more rapid onset of secretion during anoxia from the intact gland compared with the denervated preparation may indicate that in the foal the two stimuli, nervous and direct, also supplement each other so that a discharge occurs at higher P_{O_2} levels.

In view of the close relation between the growth and secretion of the adrenal cortex and the methylation of noradrenaline, particularly in the foetus (Jost, 1966; Wurtman & Axelrod, 1966; Comline, Silver & Silver, 1969), it seemed possible that the relatively high adrenaline output from the equine gland during the perinatal period might be associated with enhanced cortical development. A comparison of the changes in adrenal

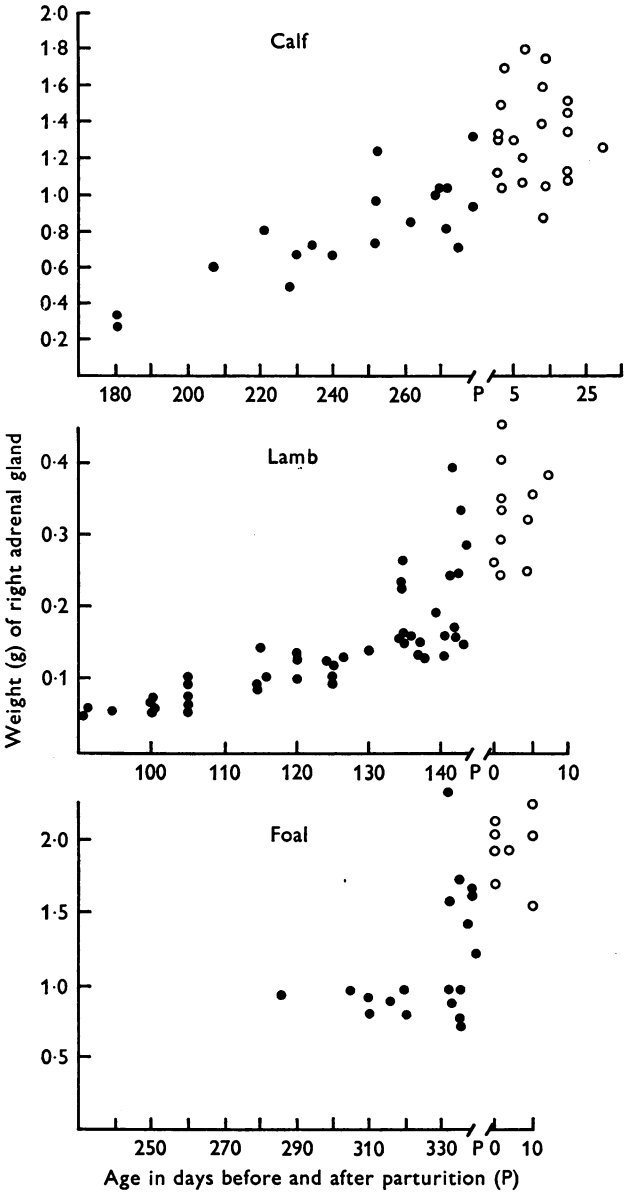

Fig. 8. Changes in foetal adrenal weight during gestation (●) and after birth (○), in the calf (Comline & Silver, 1966, plus additional data), lamb (Comline & Silver, 1961) and horse (present experiments plus additional data); P, parturition.

weight before and after birth in all three species (Fig. 8) shows that there is a very marked increase in glandular weight at term in both the lamb and the foal. In the lamb this growth is cortical not medullary (Comline & Silver, 1961; Liggins, 1969) and is accompanied by a rise in peripheral cortisol levels (Bassett & Thorburn, 1969) and utilization (Comline, Nathanielsz, Paisey & Silver, 1970). At the same time, the innervation of the gland becomes functional, adrenaline output is greatly increased during all forms of stimulation, and even the small local response to anoxia consists of 36% adrenaline. This situation in the lamb contrasts sharply with that in the foetal calf, in which cortical growth appears to be small, adrenaline secretion is minimal and the innervation of the gland immature. The position in the foal combines the apparent cortical hypertrophy of the lamb with an innervation which appears to be even more immature than that of the calf adrenal at birth. Apparently the relatively undeveloped nerve supply to the adrenal medulla of the foal is compensated by an enhanced local sensitivity of the cells. It may well be that the amounts of noradrenaline and adrenaline released during hypoxia are to some extent dependent upon the activity of the adrenal cortex, although there is little evidence for any change in the proportion of the two amines in the glands during the last month of gestation. However, the major changes in output, including a rise in the proportion of adrenaline released, occur within this period, and the secretory capacity of the gland is potentially greatest just before parturition. The low adrenaline and noradrenaline content in the new-born foal suggests depletion of the stored catecholamines during birth. Presumably the discharge is dependent on changes in P_{O_2} possibly aided by other stimuli which have yet to be specifically identified.

We are indebted to the Horserace Betting Levy Board for a grant to meet the cost of the animals. We should also like to thank the many members of the Laboratory who have helped in these experiments, and Miss Julia London for preparing the figures.

REFERENCES

BASSETT, J. M. & THORBURN, G. D. (1969). Foetal plasma corticosteroids and the initiation of parturition in the sheep. *J. Endocr.* **44**, 285–286.

BULLER, A. J. (1969). Some changes in neuromuscular functions occurring in the perinatal period. In *Foetal Autonomy*, CIBA Symposia, p. 176 London: Churchill.

COMLINE, R. S., NATHANIELSZ, P. W., PAISEY, R. B. & SILVER, M. (1970). Cortisol turnover in the sheep foetus immediately prior to parturition. *J. Physiol.* **210**, 141–142*P*.

COMLINE, R. S. & SILVER, M. (1961). The release of adrenaline and noradrenaline from the adrenal glands of the foetal sheep. *J. Physiol.* **156**, 424–444.

COMLINE, R. S. & SILVER, M. (1966). The development of the adrenal medulla of the foetal and new-born calf. *J. Physiol.* **183**, 305–340.

COMLINE, R. S. & SILVER, M. (1970). P_{O_2}, P_{CO_2} and pH levels in the umbilical and uterine blood of the mare and ewe. *J. Physiol.* **209**, 587–608.

COMLINE, R. S., SILVER, I. A. & SILVER, M. (1965). Factors responsible for the stimulation of the adrenal medulla during asphyxia in the foetal lamb. *J. Physiol.* **178**, 211–238.

COMLINE, R. S., SILVER, I. A. & SILVER, M. (1969). Effect of foetal hypophysectomy on catecholamine levels in the lamb adrenal during prolonged gestation. *Nature, Lond.* **225**, 739–740.

DAWES, G. S. (1968). *Foetal and Neonatal Physiology*. London: Year Book Medical Pub. Inc.

DIAMOND, I. & MILEDI, R. (1962). A study of foetal and new-born rat muscle fibres. *J. Physiol.* **162**, 393–408.

JOST, A. (1966). Problems of fetal endocrinology: the adrenal glands. *Recent prog. Hormone Res.* **22**, 541–569.

LIGGINS, G. C. (1969). The foetal role in the initiation of parturition in the ewe. In *Foetal Autonomy*, CIBA Symposia, p. 218. London: Churchill.

REDFERN, P. A. (1970). Neuromuscular transmission in new-born rats. *J. Physiol.* **209**, 701–709.

ROSSDALE, P. D. (1967). Clinical studies on the newborn thoroughbred foal. 1. Perinatal behaviour. *Br. vet. J.* **123**, 470–481.

SILVER, M. (1960). The output of adrenaline and noradrenaline from the adrenal glands of the calf. *J. Physiol.* **152**, 14–29.

WURTMAN, R. J. & AXELROD, J. (1966). Control of enzymatic synthesis of adrenaline in the adrenal medulla by adrenal cortical steroids. *J. biol. Chem.* **214**, 2301–2305.